Serial No. : 09/245,549

Filed : February 5, 1999

Page : 2 of 13

## Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

## Listing of Claims:

1. (currently amended) A method for constructing a DNA library in vivo, comprising: providing a plurality of host cells;

providing a vector molecule having a first region and a second region;

providing a plurality of nucleic acid insert molecules, each of the plurality of nucleic acid insert molecules having a first common region which is homologous with said first region of the vector molecule, a second common region which is homologous with said second region of the vector molecule, and a library element encoding region disposed between said first common region and said second common region, wherein when the library element encoding region encodes a naturally occurring sequence, the first and second regions are not naturally found adjacent to the library element encoding region;

introducing the vector molecule into each host cell of the plurality of host cells; introducing a nucleic acid insert molecule from said plurality of nucleic acid insert molecules into each host cell of said plurality of host cells, wherein a different library element encoding region is introduced into each host cell of said plurality of host cells;

allowing homologous recombination and gap repair between a vector molecule and a <u>only one</u> nucleic acid insert molecule to occur, and

generating a plurality of vector molecules from said plurality of host cells, each vector molecule of the plurality comprising a different nucleic acid insert library element encoding region, and each vector comprising only one library element encoding region,

Serial No.: 09/245,549 Filed: February 5, 1999

Page : 3 of 13

thereby constructing a DNA library.

## 2. (canceled)

3. (currently amended) A method of constructing a DNA library, comprising: providing a plurality of nucleic acid molecules wherein each of said nucleic acid molecule includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region which hybridizes to the first common sequence of the nucleic acid molecule and having a second region which does not hybridize to said first or second common sequence;

providing a plurality of second primers, each of said second primers having a first region which hybridizes to the second common sequence of the nucleic acid molecule and having a second region which does not hybridize to said second or first common sequence;

forming a reaction mixture which includes said plurality of nucleic acid molecules, said plurality of said first primers, and said plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of said first primer/said first common region/a library element encoding region/said second common region/a second region of said second primer;

providing a plurality of host cells;

providing a vector molecule having a first region which is homologous with said second region of said first primer, and a second region which is homologous with said second region of said second primer;

introducing said vector molecule into each host cell of the plurality of host cells;

Serial No.: 09/245,549 Filed: February 5, 1999

Page : 4 of 13

introducing one or more of nucleic acid insert molecules from said plurality of nucleic acid insert molecules into each host cell of the plurality of host cells;

allowing homologous recombination and gap repair to occur between a vector molecule and only one nucleic acid insert molecule; and

generating a plurality of vector molecules from said plurality of host cells, each vector molecule of the plurality comprising a different nucleic acid insert molecule library element encoding region, and each vector comprising only one library element encoding region,

thereby providing a DNA library.

- 4. (canceled)
- 5. (original) The method of claim 3, wherein said first and second common sequences are the same.
- 6. (original) The method of claim 3, wherein said first and second common sequences are different.
- 7. (previously presented) The method of claim 1, wherein said host cell is a yeast cell.
- 8. (previously presented) The method of claim 1, wherein said host cell is a bacterial cell.
- 9. (previously presented) The method of claim 1, wherein said at least one vector is linearized prior to being introduced into said host cell.

Serial No.: 09/245,549 Filed: February 5, 1999

Page : 5 of 13

10. (original) The method of claim 9, wherein said vector is linearized by cleaving between said first and second regions of said vector.

- 11. (previously presented) The method of claim 1, wherein said second common region of said nucleic acid insert molecule is produced by PCR.
- 12. (previously presented) The method of claim 1, wherein said first common region of said nucleic acid insert molecule is produced by PCR.
- 13. (previously presented) The method of claim 1, wherein said second common region of said nucleic acid insert molecule is produced by the ligation of adapters.
- 14. (previously presented) The method of claim 1, wherein said first common region of said nucleic acid insert molecule is produced by the ligation of adapters.
- 15. (previously presented) The method of claim 1, wherein said first and second common regions of said nucleic acid insert molecule are at least 30 base pairs in length.
- 16. (previously presented) The method of claim 1, wherein said first and second regions of said nucleic acid insert molecule are at least 40 base pairs in length.
- 17. (previously presented) The method of claim 1, wherein said first and second regions of said nucleic acid insert molecule are at least 50 base pairs in length.
- 18. (previously presented) The method of claim 1, wherein said library element encoding region is obtained from a cDNA library other than the one being constructed.

Serial No.: 09/245,549

Filed: February 5, 1999

Page : 6 of 13

19. (original) The method of claim 18, wherein said library element encoding region is obtained from a cDNA library which is plasmid based.

- 20. (original) The method of claim 18, wherein said library element encoding region is obtained from a cDNA library which is phage based.
- 21. (previously presented) The method of claim 1, wherein said library element encoding region is obtained from an mRNA molecule.
- 22. (original) The method of claim 21, wherein said mRNA molecule is obtained from a cancerous tissue.
- 23. (previously presented) The method of claim 1, wherein said DNA library is screened in a two-hybrid system and wherein said vector includes a transcription factor activation domain.
- 24. (previously presented) The method of claim 23, wherein said method further comprises,

introducing into each host cell of said plurality of host cells a nucleic acid molecule encoding a hybrid protein, wherein the hybrid protein comprises a transcription factor DNA-binding domain attached to a test protein;

introducing into each host cell of said plurality of host cells a detectable gene, wherein said detectable gene comprises a regulator site recognized by said DNA-binding domain and wherein said detectable gene expresses a detectable protein when said test protein interacts with a protein encoded by the DNA library;

plating each host cell of said plurality of host cells onto selective media; and selecting for each host cell of said plurality of host cells containing a DNA encoded protein which interacts with test protein.

Serial No.: 09/245,549

Filed: February 5, 1999

Page : 7 of 13

25. (previously presented) The method of claim 1, wherein said DNA library is used for screening and cloning of novel genes.

26. (currently amended) A method of constructing a DNA library for screening in a two-hybrid system, comprising:

providing a plurality of nucleic acid molecules, wherein each of the plurality of nucleic acid molecules includes, in order from 5' to 3', a first common sequence, a library element encoding region, and a second common sequence;

providing a plurality of first primers, each of said first primers having a first region which hybridizes to said first common sequence of said nucleic acid molecule and having a second region which does not hybridize to said first or second common sequence;

providing a plurality of second primers, each of said second primers having a first region which hybridizes to said second common sequence of said nucleic acid molecule and having a second region which does not hybridize to said second or first common sequence;

forming a reaction mixture which includes the plurality of nucleic acid molecules, the plurality of said first primers, and the plurality of said second primers, under conditions which provide a plurality of nucleic acid insert molecules having the following structure, in order from 5' to 3', a second region of the first primer/the first common region/a library element encoding region/the second common region/a second region of the second primer;

providing a plurality of host cells;

providing a vector having a first region which is homologous with the second region of the first primer, and a second region which is homologous with the second region of the second primer, wherein said vector further includes a transcription factor activation domain;

introducing a the vector molecule into each of each host cell of said plurality of host cells;

Applicant: Zervos et al.
Serial No.: 09/245,549
Filed: February 5, 1999

Page : 8 of 13

introducing one or more of the nucleic acid insert molecules into each host cell of said plurality of host cells under conditions which allow for recombination and gap repair to occur;

allowing homologous recombination and gap repair to occur between a vector molecule and only one nucleic acid insert molecule in each host cell of the plurality of host cells, wherein each host cell of the plurality comprises a vector molecule that comprises (a) a different nucleic acid insert molecule library element encoding region, and (b) only one library element encoding region,

introducing into each host cell of said plurality of host cells a nucleic acid molecule encoding a hybrid protein, wherein the hybrid protein includes a transcription factor DNA-binding domain attached to a test protein;

introducing into each host cell of said plurality of host cells a detectable gene, wherein said detectable gene comprises a regulator site recognized by the DNA-binding domain and wherein said detectable gene expresses a detectable protein when the test protein interacts with a protein encoded by the DNA library;

plating each host cell of said plurality of host cells onto selective media; and selecting for each host cell of said plurality of host cells containing a DNA encoded protein which interacts with test protein.

27. (canceled)